EXHBIT H

cells contain only about 30% of the cellular RNA content of the larger MPC-11 cells, and we have accommodated this by loading RNA extracted from equal numbers of cells of both types (Fig. 4): MPC-11 in lane 1, 38c in lane 2. Lane 3, in Fig. 4 contains 5 times that equivalent of 38c. The quantitative difference in κ gene transcripts between the two cell types is very obvious; it seems however, to apply roughly equally to all transcript classes: the ratio of primary mRNA precursors to excised introns appears to be about the same in both cell lines.

DISCUSSION

We have investigated the properties of a hitherto undescribed class of immunoglobulin gene transcripts. These molecules lack poly(A), hybridize with intron probes, but not with exon probes, have apparent lengths similar to known intronic lengths, characteristically migrate as doublet bands on electrophoresis, and have a subcellular distribution distinct from that of the poly(A)$^+$ mRNA precursors. In S_1 nuclease mapping experiments of plasmacytoma RNA, we have not detected any molecules having termini or sequence interruptions in the κ intron (Wood and Coleclough, in press; unpublished results). These properties distinguish these RNA molecules from other κ gene transcripts and establish them as free introns, which we presume derive from the splicing of mRNA precursors. As mentioned above, we were initially alerted to the existence of this class of immunoglobulin gene transcript by the anomalous results of a preliminary S_1 mapping study of heavy chain RNA. (A more complicated situation, which we have not further addressed, may obtain for heavy-chain gene transcripts in that excised introns seem to undergo additional processing, unlike the κ introns described here.) If introns excised from both κ and heavy-chain immunoglobulin mRNA precursors persist significantly in plasma cell RNA in the steady state, is this the case for introns in all genes? Chambon et al. (3) reported the existence in chicken oviduct cells of RNA molecules that might well be the excised form of one of the ovalbumin gene introns. On the other hand, a study of late adenovirus transcripts, which should have revealed free introns (if they exist) spliced out from the triparite leader RNA sequence, failed to do so (1), and we have been unable to detect introns excised from precursors of a ribosomal protein mRNA expressed in plasmacytoma cells (Coleclough, unpublished data). It must be emphasized that many studies of mRNA precursors, unless designed purposefully, might not include excised introns, which must be sought in unfractionated or poly(A)$^-$ RNA with intron probes and may actually be depleted from carefully washed nuclei. Nonetheless, it is our impression that the intronic forms described here for immunoglobulin genes may not be detectable in the steady state for all other nuclear genes.

We have interpreted the electrophoretic doublet that free introns produce as indicating the coexistence of linear and circular forms. Conclusive proof of circular introns will depend on demonstrating a continuous sequence spanning the 5' and 3' ends of the linear intron sequence, data which we are attempting to obtain. Moreover, it is important to determine whether the linear form is colinear with the gene sequence or a circular permutation of it. Such information may help decide whether the immediate excision product is linear or circular. Thus, the linear form may be the precursor of the circular one, or vice versa, or conceivably they could even be products of different splicing mechanisms.

The existence of the 4.1-kb poly(A)$^+$ species, cleaved at the 5' intron-exon junction, indicates that strand scission can be divorced from ligation. Interestingly, we failed to detect a symmetrical cleavage product, nicked at the 3' end of the intron; perhaps this reflects the greater affinity of 5' intron-exon junction sequences for U_1-ribonucleoprotein particles (14) and suggests that cleavage at the 3' intron-exon boundary might be rate limiting for splicing.

That excised introns are extractable from nuclei with nonionic detergents is a provocative observation, suggestive of a connection between the splicing process and the transfer of spliced products from the nucleus to the cytoplasm. An obligatory coupling between intron excision and export from the nucleus is unlikely, however, since the production of most messengers requires the removal of multiple introns. It is possible that if the final intron to be removed from a messenger is somehow earmarked, its excision could be coupled with vectorial transfer of mRNA. The distribution of free introns may simply indicate an association of the mRNA-splicing apparatus with the nuclear membrane, although this is not true of components capable of t-RNA splicing in *Xenopus* oocytes (8). Similarly the preferential retention by nuclei of the higher-mobility intron form, which we take to be linear, suggests some connection between the nuclear membrane and an activity capable of cyclizing introns, although again there might exist separate nucleoplasmic and nuclear membrane-splicing systems. Alternatively, solubilization of the nuclear membrane may allow the escape of nucleoplasmic species, whereas those closely associated with chromatin or other structural components are retained by the nucleus.

The implications of our observations depend on whether these phenomena will be seen for all genes specifying spliced messengers. We assume that the excision of introns is largely similar in all cases, so that what may be remarkable about these immunoglobulin introns is their persistence. These molecules are nonetheless ephemeral, having half-lives probably of only a few minutes. Initially we thought that the persistence of κ introns might be symptomatic of some special mechanism for splicing κ mRNA precursors—a process which must avoid the use of splicing signals for J elements 3' of that adjacent to a V gene. However excised introns are also detectable from the aberrantly rearranged κ locus of MPC-11, where splicing does not involve any J segment. Neither is the persistence of κ introns due to the rapid flux of κ gene transcripts through the processing machinery, necessary to maintain the high level of expression seen in plasmacytomas; they seem to be equally persistant in lymphoma cells maintaining a much lower level of κ gene expression. Future experiments will help decide whether these free introns are simply inert processing by-products or whether they do have some informational role to play.

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